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Ltd., Ten South Wacker Drive, Chicago, IL 60606 (US).**(81) Designated States: **AT, AU, BB, BG, BR, CA, CH, CZ,
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SN, TD, TG).****Published***With international search report.**Before the expiration of the time limit for amending the
claims and to be republished in the event of the receipt of
amendments.*(54) Title: **SELF-STABILIZED OLIGONUCLEOTIDES AS THERAPEUTIC AGENTS**

(57) Abstract

The invention provides improved antisense oligonucleotides that are resistant to nucleolytic degradation. Such oligonucleotides are called self-stabilized oligonucleotides and comprise two regions: a target hybridizing region having a nucleotide sequence complementary to a nucleic acid sequence that is from a virus, a pathogenic organism, or a cellular gene; and a self-complementary region having an oligonucleotide sequence complementary to a nucleic acid sequence that is within the self-stabilized oligonucleotide.

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SELF-STABILIZED OLIGONUCLEOTIDES AS THERAPEUTIC AGENTS.

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BRIEF SUMMARY OF THE INVENTION

The invention relates to novel therapeutic agents used in the antisense oligonucleotide therapeutic approach. The invention provides improved antisense oligonucleotides that are resistant to nucleolytic degradation.

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The self-complementary region comprises an oligonucleotide sequence that is complementary to a nucleic acid sequence within the oligonucleotide. Thus, at least when the oligonucleotide is not hybridized to a target nucleic acid sequence, the oligonucleotide forms a totally or partially double-stranded structure that is resistant to nucleolytic degradation.

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5 The second significant region of self-stabilized
oligonucleotides according to the invention is the self-
complementary region. The self-complementary region
contains oligonucleotide sequences that are complementary
to other oligonucleotide sequences within the
oligonucleotide. These other oligonucleotide sequences
may be within the target hybridizing region or within the
self-complementary region, or they may span both regions.
10 The complementary sequences form base pairs, resulting in
the formation of a hairpin structure, as shown in Figure
1, or a hammer-like structure, as shown in Figure 2.
Either the hairpin structure or the hammer-like structure
can have loops resulting from non-base-paired
nucleotides, as shown in Figure 1 for the hairpin
15 structure, or can be devoid of such loops, as shown in
Figure 2 for the hammer-like structure.

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Of course, the
intra-molecular base-pairing can be so extensive as to
involve every nucleotide of the oligonucleotide.
Preferably, this will involve a self-complementary region
30 of about 50 nucleotides or less.

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10 All self-stabilized oligonucleotides exhibited greater anti-HIV activity than CMPD A, the non-self-stabilized oligonucleotide. Greatest activity was observed for the self-stabilized oligonucleotide having 10 self-complementary nucleotides, which exhibited nearly ten times the activity of the oligonucleotide phosphodiester. The oligonucleotide CMPD G, which has a poly T tail, also showed some increase in activity,

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To test the relative anti-HIV activity of additional oligonucleotide structures, the above experiment was repeated using additional oligonucleotides, as well as the oligonucleotides described in Experiments 1 & 2. The additional oligonucleotides are shown in Figure 5. These additional oligonucleotides were CMPD C, in which the self-complementary region is complementary to the oligonucleotide through its 5' end; CMPD D, which has a 8 nucleotide self-complementary region; and CMPD H, a 35 mer oligonucleotide having perfect complementarity to the HIV gag RNA, but no self-complementary region. The results of this third experiment are shown in Table IV, below.

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These results demonstrate that fully self-complementary self-stabilized oligonucleotides are roughly equivalent in anti-HIV activity to partially self-complementary self-stabilized oligonucleotides.

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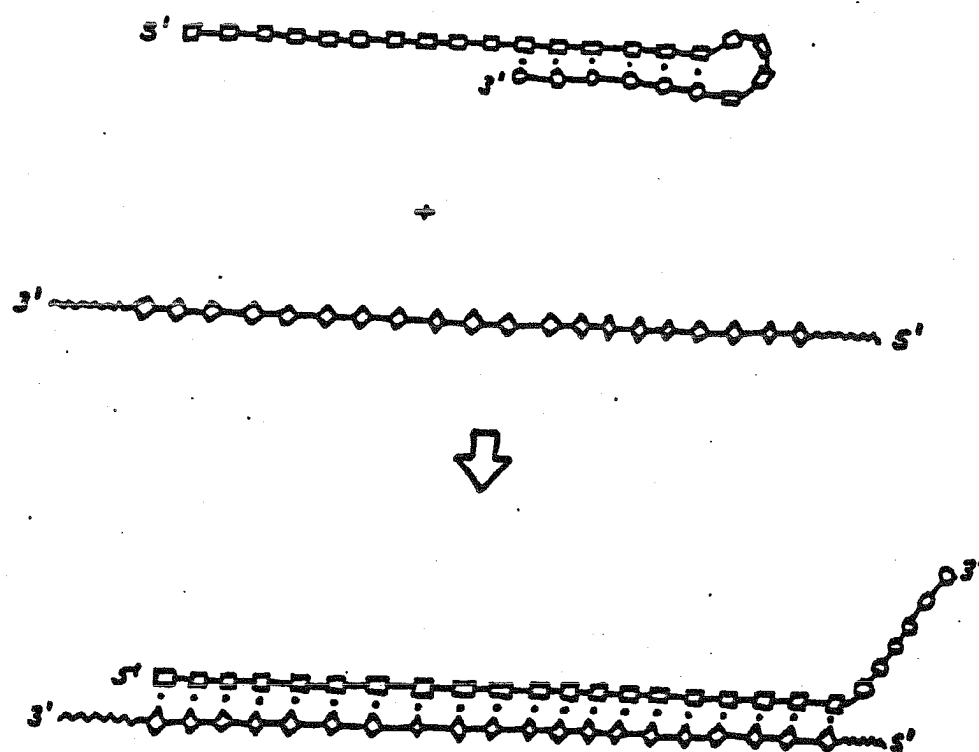
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Fig. 1

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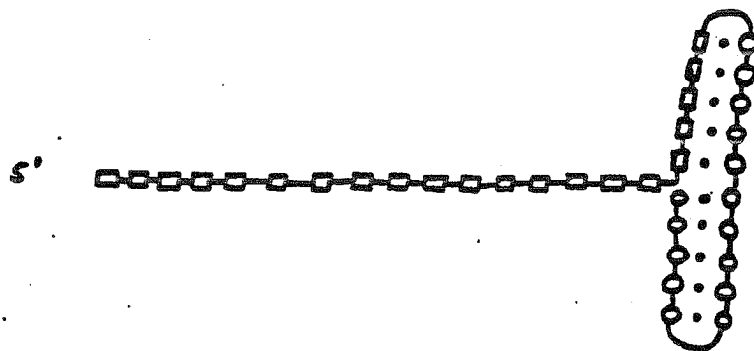
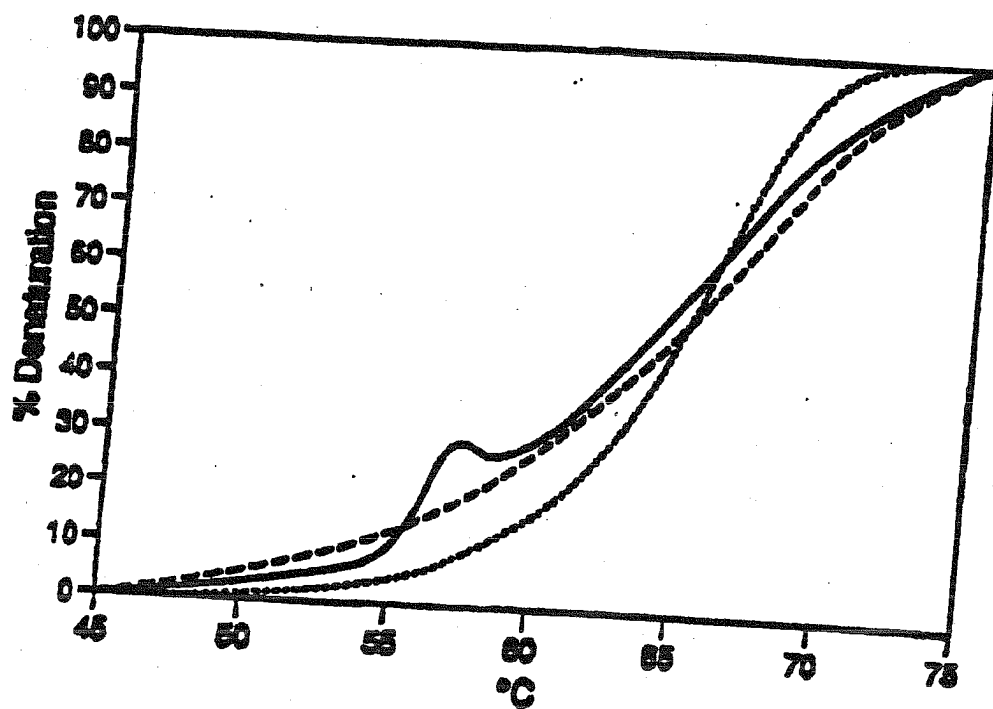


Fig. 2

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Fig.3

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Fig. 4

CMPD A	CMPD B	CMPD C	PO	(min)
0	0	0	0	0
30	30	30	30	30
60	60	60	60	60
120	120	120	120	120

CMPD A	CMPD B	CMPD C	CMPD F	CMPD G
0	0	0	0	0
30	30	30	30	30
60	60	60	60	60
120	120	120	120	120

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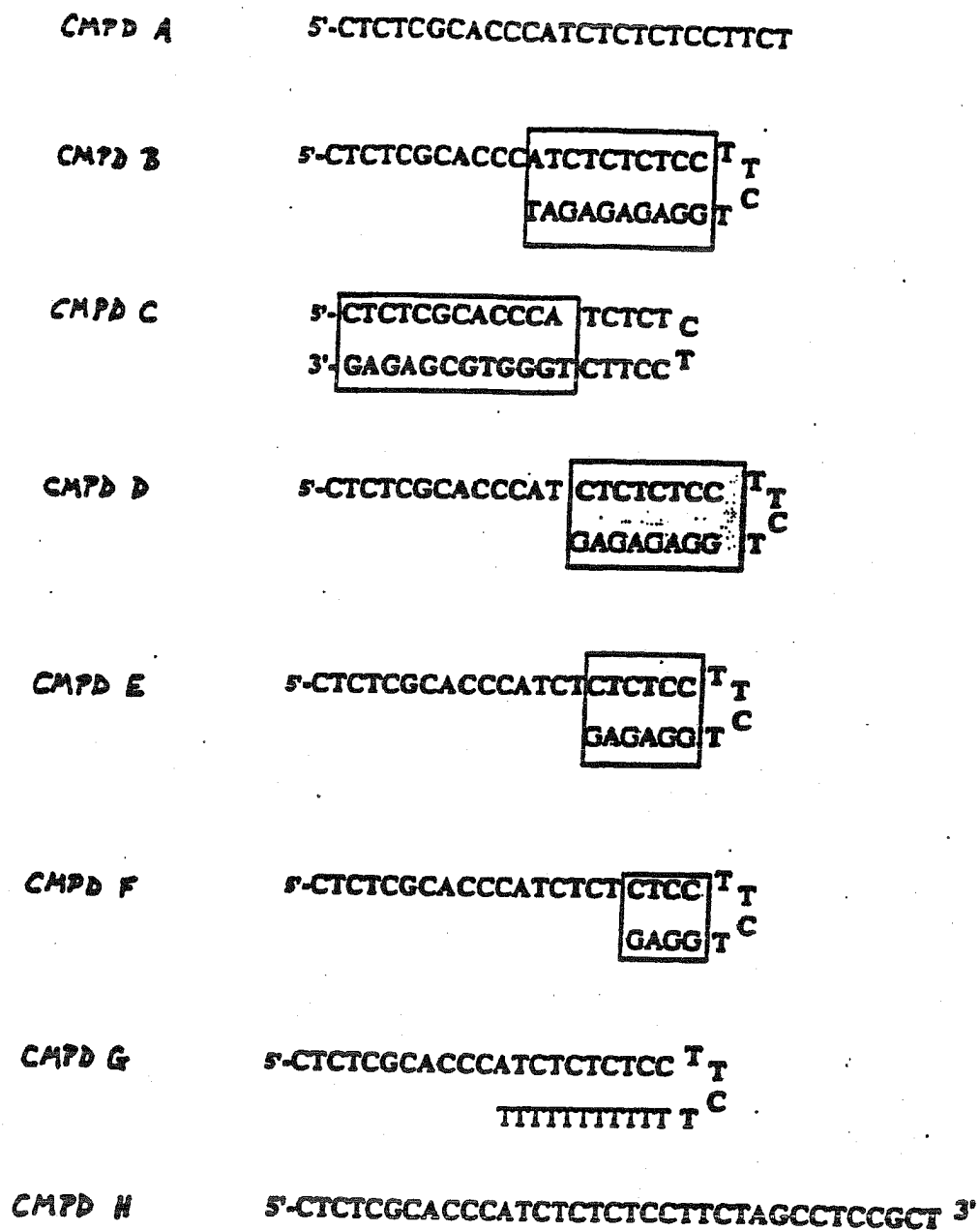


Fig. 5

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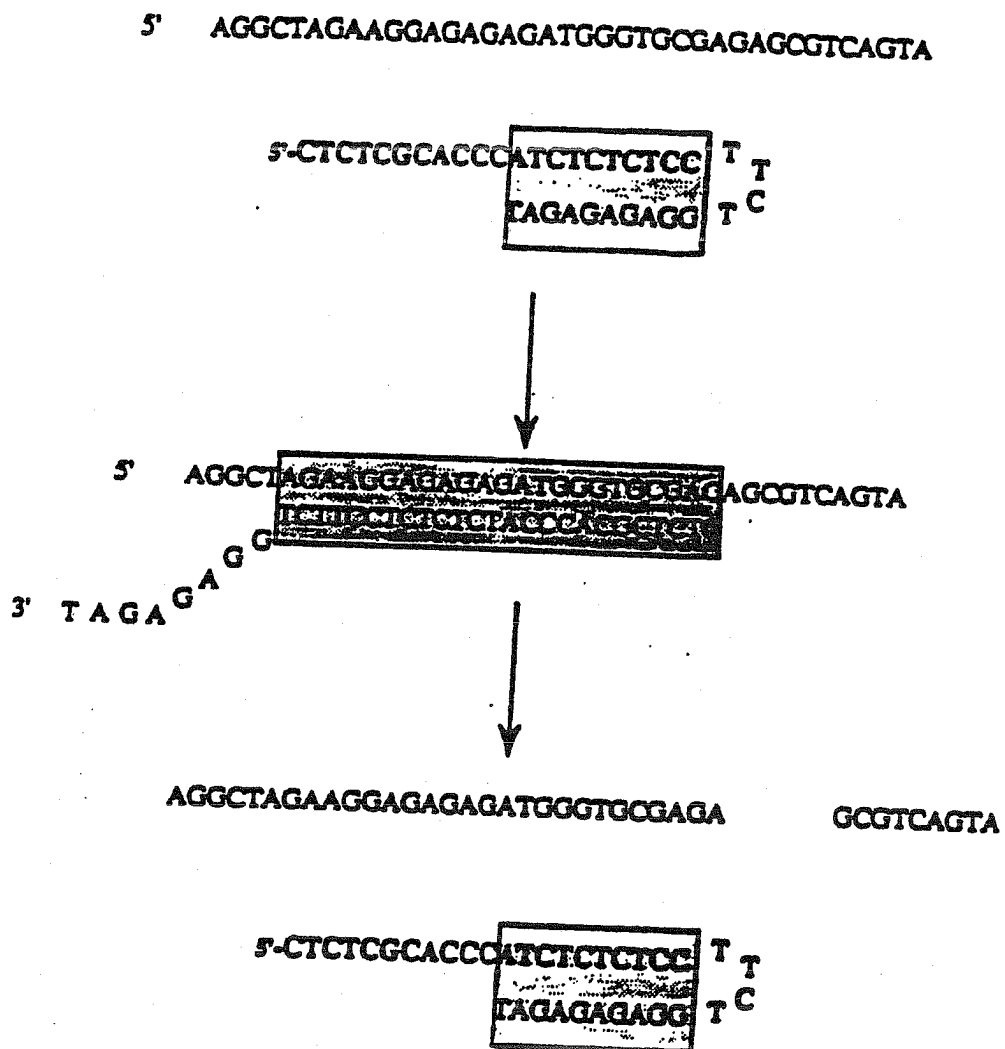


Fig. 6

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 93/06326

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 5 C12N15/11 C07H21/00 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C12N C07H A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EMBO JOURNAL vol. 8, no. 13, 1989, EYNSHAM, OXFORD GB pages 4297 - 4305 CASE C.C. ET AL 'The unusual stability of the IS10 anti-sense RNA is critical for its function and is determined by the structure of the stem-domain' see page 4303, column 2	1-21
Y	---	22
Y	WO,A,92 03464 (MICROPROBE CORPORATION) 5 March 1992 see claims; example 32 ---	22
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

16 November 1993

Date of mailing of the international search report

30. 11. 93

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	NUCLEIC ACIDS RESEARCH vol. 21, no. 11 , 11 June 1993 , ARLINGTON, VIRGINIA US pages 2729 - 2735 TANG J.Y. ET AL 'Self-stabilized antisense oligodeoxynucleotide phosphorothioates: properties and anti-HIV activity'	1-21
P,Y	see page 2733, column 2, line 20 - page 2735	22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/06326

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 18-20 and claims 15-17(partially) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
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4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 93/06326

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9203464	05-03-92	EP-A- 0547142	23-06-93